Veterinary Parasitology 185 (2012) 305–308

View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

ELSEVIER

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Factors associated with infection by *Neospora caninum* in dogs in Brazil

Patrícia Mara Lopes Sicupira[a], Vanessa Carvalho Sampaio de Magalhães[a], Gideão da Silva Galvão[b], Maria Julia Salim Pereira[c], Luís Fernando Pita Gondim[d], Alexandre Dias Munhoz[a,*]

[a] *Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz (UESC), Campus Soane Nazaré de Andrade Rodovia Ilhéus Itabuna, Km 16, Salobrinho, Ilhéus, Bahia 45662-000, Brazil*

[b] *Curso de Pós-graduação em Ciências Veterinárias, Instituto de Veterinária-IV Universidade Federal Rural do Rio de Janeiro (UFRRJ) BR-465 Km 7 Seropédica, Rio de Janeiro, Brazil*

[c] *Departamento de Parasitologia Animal, IV, UFRRJ BR-465 Km 7 Seropédica, Rio de Janeiro, Brazil*

[d] *Departamento de Patologia e Clínicas, Escola de Medicina Veterinária, Universidade Federal da Bahia, Av. Ademar de Barros 500, Ondina, Salvador, Bahia 40170-110, Brazil*

ARTICLE INFO

Article history:
Received 21 April 2011
Received in revised form
15 September 2011
Accepted 20 September 2011

Keywords:
Neosporosis
Risk factors
Canines

ABSTRACT

From August 2006 to 2008, 411 dogs in northeastern Brazil were evaluated for seropositivity to *Neospora caninum*. The dogs were clinically examined, and their owners were interviewed about the conditions in which the animals were maintained in order to assess the factors associated with infection by this parasite. A serum sample was taken from each dog for serological examination in an indirect fluorescent antibody test for *N. caninum*. The Yates' Chi-square test or Fisher's exact test was used to select the variables for the multivariate logistic regression model. Seropositivity was detected in 9.26% of dogs. The seropositivity rates of dogs from different environments were 2.6% (4/156) in urban areas, 13.1% (28/214) in peri-urban areas, and 14.6% (6/41) in rural areas. Factors associated with seropositivity for *N. caninum* were the following: contact with other dogs, access to food outside the home and residing in the peri-urban or rural environments ($p < 0.05$). Results of this study confirm that dogs in urban, rural and peri-urban areas of northeastern Brazil are exposed to *N. caninum*. Control measures to prevent infection of dogs in the studied region should be focused primarily on preventing access to potential sources of infection, which include environments with other dogs, bovines, and other small intermediate hosts, such as birds and rodents.

© 2011 Elsevier B.V. Open access under the Elsevier OA license.

1. Introduction

Neospora caninum is an apicomplexan protozoan that belongs to the family Sarcocystidae. This protozoan is structurally very similar to, but antigenically distinct from, *Toxoplasma gondii* (Hemphill et al., 1999), and its definitive hosts are the dog (Mcallister et al., 1998), the coyote (Gondim et al., 2004) and the dingo (King et al., 2010).

Dogs of any age can develop clinical signs of neosporosis (Dubey, 2003); yet, in most cases, hosts are asymptomatic (Pasquali et al., 1998). The infection of dogs is observed throughout the world, and ingestion of any of the following is associated with infection: rodents, birds and other animals (Jesus et al., 2006) that can be intermediate hosts of *N. caninum* (Wouda et al., 1999; Costa et al., 2008); improperly cooked meat or homemade food (Cañón-Franco et al., 2003; Ferroglio et al., 2007); and most likely the direct ingestion of oocysts. Additionally, contact between cattle and dogs in rural areas allows for the ingestion of fetal membranes and fluids from infected cattle (Wouda et al., 1999). These factors contribute to horizontal transmission of the

* Corresponding author. Tel.: +55 73 3680 5406; fax: +55 73 3680 5406.
E-mail addresses: munhoz@uesc.br, admruralvet@ig.com.br (A.D. Munhoz).

0304-4017 © 2011 Elsevier B.V. Open access under the Elsevier OA license.
doi:10.1016/j.vetpar.2011.09.029

parasite in dogs, which is the primary route of infection because the rate of vertical transmission is low and insufficient to maintain the infection in the canine population (Barber and Trees, 1998).

The studies conducted to date indicate that factors associated with infection by *N. caninum* vary by region and with the management conditions of the dogs. Thus, this study aimed to evaluate *N. caninum* infection and the factors associated with parasite seropositivity in dogs from different environments in northeastern Brazil.

2. Materials and methods

The present study was conducted from August 2006 to 2008 in the city of Ilhéus (14°47′ latitude and 39°02′ longitude), Bahia State, in the northeastern region of Brazil. A non-probabilistic sample of 411 dogs from urban, peri-urban and rural (farm dog) areas of the region was analyzed. Animals included in the study were clinically examined after receiving the written consent of their owners, who were interviewed about the conditions of the dogs, with an emphasis on management, feeding habits, contact with other animals, hunting habits, and access to the streets (Table 1).

The sera were tested for antibodies to *N. caninum* by an indirect fluorescent antibody test using the NC-1 parasite strain (Dubey et al., 1988). Commercial FITC-labeled anti-dog IgG (Sigma–Aldrich) was used as a secondary antibody. The serum dilution threshold for positivity was set at 1:50. Negative and positive controls consisted of pre and post-infection sera from a dog that received bovine tissue containing cysts of *N. caninum* (Gondim et al., 2005).

Database and statistics software Epi Info version 3.5.1 (Centers for Disease Control and Prevention) were used to perform the analyses of the association between the explanatory variables and the infection. The bivariate analysis was carried out using the Yates' Chi-square (χ^2) test, or the Fisher's exact test, whenever necessary. Before further analysis, the R Program (The R Project for Statistical Computing, http://www.r-project.org) for Windows version 2.10.1 was employed to perform Spearman's correlation matrix in order to verify the multicollinearity among the independent variables ($\rho \geq 0.80$). The variables with $p \leq 0.20$ in the bivariate analysis and $\rho < 0.80$ in the correlation analysis were included in the multivariable analysis by logistic regression. The variable "rural environment" was included in the model due to its biological relevance despite its failure to achieve statistically significant association in the bivariate analysis (Katz, 1999). The variables introduced in the model were selected through the *backward elimination* procedure based on the likelihood-ratio test. The required level of significance for a factor to be considered as associated in the final model was set at $p < 0.05$.

3. Results

Antibodies against *N. caninum* were detected in 38/411 (9.26%, 95% CI: 6.7–12.6) of the dogs. Eight dogs (21.1%, 95% CI: 9.6–37.3) had titers of 50, 12 (31.6%, 95% CI: 17.5–48.7) had titers of 100, and 18 (47.4%, 95% CI: 31–64.2) had titers of 200. The seropositivity rates for dogs from different environments were 4/156 (2.6%, 95% CI: 0.7–6.4) in urban areas, 28/214 (13.1%, 95% CI: 8.9–18.4) in peri-urban areas, and 6/41 (14.6%, 95% CI: 5.6–29.2) in rural areas. No animals showed clinical symptoms of infection.

Results of the bivariate analysis are shown in Table 1. The variable rural environment ($p = 0.33$) did not meet the inclusion criteria, however, it has been included in the model because it was epidemiologically important. Due to the correlation ($\rho \geq 0.80$) between the variables urban and peri-urban environment, peri-urban was chosen to be part of the analysis because the majority of animals lived in that environment.

In the final model, factors such as contact with other dogs, access to food outside the home, and residence in peri-urban or rural environments remained significantly associated with infection by *N. caninum* (Table 2).

4. Discussion

The absence of clinical signs in the seropositive dogs has been observed in other epidemiological studies (Cañón-Franco et al., 2003; Collantes-Fernández et al., 2008). Barber and Trees (1996) suggest that high antibody titers against *N. caninum* (>800) can be strong evidence of clinical neosporosis; our findings reinforce the observation that high antibody concentrations rarely occur in asymptomatic animals, in which we found maximum titers to be 200. However, Gondim et al. (2005) and Romanelli et al. (2007) have observed dogs with antibody titers higher than 800 without clinical manifestations of the disease, and Basso et al. (2001) have not observed differences in antibody titers between symptomatic and asymptomatic animals. Thus, repeated exposures to the parasite are likely to elevate antibody titers without manifesting in overt disease.

Although contact with cattle was a significant factor in the bivariate analysis and is epidemiologically important (Kramer et al., 2004), it was not included in the model due to the insufficient number of samples in one of the categories (Table 1). Thus, contact of dogs with cattle may be a confounding factor in our model. For dogs residing in urban and peri-urban areas, seropositivity to *N. caninum* was significantly greater for those in contact with cattle (data not shown). In rural environments, however, 39 of 41 dogs had contact with cattle and six of these 39 were seropositive. Neither of the two dogs without contact with cattle was seropositive.

In this study, 29/34 (85.3%, 95% CI: 68.9–95.0) of seropositive dogs from rural and peri-urban environments had contact with other dogs. Thus, due to the dog–bovine–dog cycle of parasite transmission (Dijkstra et al., 2001), dogs from rural areas may have a greater chance of ingestion of oocysts present in the environment, though firm evidence of this route of infection is lacking. Alternatively, dogs from peri-urban areas are often semi-residents, i.e., despite having owners, they have access to the streets and are in contact with stray dogs. These semi-resident dogs are therefore exposed to oocysts dispersed in the environment. Furthermore, because all seropositive dogs from rural and peri-urban environments had contact with cattle, the possibility of having ingested placental

Table 1
Factors assessed using the Chi-square test in order to evaluate association with infection by *Neospora caninum* in dogs from Ilhéus, State of Bahia, Brazil.

Factors	Category	Positive dogs *n* (%)	Negative dogs *n* (%)	Odds ratio (95% CI)	*p*
Access to street	Yes	30 (11.4)	234 (88.6)	2.23 (0.99–5.00)	0.070
	No	8 (5.4)	139 (94.6)	*[a]	
Hunting habits	Yes	14 (18.7)	61 (81.3)	2.98 (1.46–6.09)	0.004
	No	24 (7.1)	312 (92.9)	*	
Urban environment	Yes	4 (2.6)	152 (97.4)	0.17 (0.05–0.52)	0.0005
	No	34(13.3)	221 (86.7)	*	
Rural environment	Yes	6 (14.6)	35 (85.4)	1.81 (0.7–4.63)	0.33
	No	32 (8.6)	338 (91.4)	*	
Peri-urban environment	Yes	28 (13.1)	186 (86.9)	2.81 (1.33–5.96)	0.009
	No	10 (5.1)	187 (94.9)	*	
Contact with other dogs	Yes	31 (13.6)	197 (86.4)	3.96 (1.61–10.13)	0.0013
	No	7 (3.8)	176 (96.2)	*	
Ingestion of milk	Yes	21 (14.2)	127 (85.8)	2.39(1.22–4.70)	0.016
	No	17 (6.5)	246 (93.5)	*	
Access to food outside the home	Yes	24 (13.0)	160 (87.0)	2.28(1.14–4.55)	0.026
	No	14 (6.2)	213 (93.8)	*	
Age <12 months	Yes	1 (1.9)	51 (98.1)	0.17(0.023–1.27)	0.070[b]
	No	37 (10.3)	322 (87.3)	*	
Eats homemade food	Yes	32 (9.4)	308 (90.6)	1.13 (0.45–2.80)	0.98
	No	6 (8.5)	65 (91.5)	*	
Contact with chickens	Yes	4 (10.5)	34 (89.5)	1.17 (0.39–3.50)	0.99
	No	34 (9.1)	339 (90.9)	*	
Defined breed	Yes	4 (7.3)	51 (92.7)	0.74 (0.25–2.18)	0.77
	No	34 (9.6)	322 (90.4)	*	
Sex	Female	10 (7.7)	120 (92.3)	0.75 (0.35–1.60)	0.57
	Male	28 (10.0)	253 (90.0)	*	
Eats bovine meat	Yes	34 (9.9)	308 (90.1)	1.79 (0.62–5.23)	0.39
	No	4 (5.8)	65 (94.2)	*	
The meat provided is cooked	Yes	29 (9.7)	269 (90.3)	0.82 (0.32–2.09)	0.87
	No	6 (11.5)	46 (88.5)	*	
Contact with bovines	Yes	36 (34.0)	70 (66.0)	77.91(18.32–331.31)	<0.00001
	No	2 (0.7)	303 (99.3)	*	

[a] Reference category.
[b] Fisher's exact test.

membranes, an important source of *N. caninum* infection (Dijkstra et al., 2001) is increased.

Dogs that fed outside of their residences had higher rates of seropositivity, probably due to the possibility of ingesting contaminated food or tissue cysts in rodents, birds and other intermediate hosts of *N. caninum* (Costa et al., 2008). This variable could explain the higher positivity rates observed in dogs from rural and peri-urban areas compared to dogs from urban environments, in accordance with studies by Wouda et al. (1999), Ferroglio et al. (2007), Collantes-Fernández et al. (2008) and Cruz-Vazquez et al., 2008.

Despite evidence of horizontal parasite transmission, no differences among age groups could be statistically confirmed (Table 2). The small sample size of seropositive animals younger than 12 months is likely to have influenced this result. In the literature, there is no consensus on age as a factor associated with the infection of dogs; some studies show no association with age (Jesus et al., 2006; Romanelli et al., 2007), while others consider age a risk factor (Barber and Trees, 1998; Collantes-Fernández et al., 2008; Cruz-Vazquez et al., 2008). These disparities may be due to the failure of some infected animals to seroconvert or to slow seroconversion that takes several months in some animals (Gondim et al., 2005).

Results of this study confirm that dogs in urban, rural and peri-urban areas of northeastern Brazil are exposed to *N. caninum*. Control measures to prevent infection of dogs in the studied region should be focused primarily on preventing access to potential sources of infection, which

Table 2
Final multivariable logistic regression model for *Neospora caninum* infection in dogs from Ilhéus, State of Bahia, Brazil.

Factors	Odds ratio	95% CI	*p*
Contact with other dogs (Yes/No)	3.4056	1.4223–8.1545	0.0059
Age < 12 months (Yes/No)	0.1404	0.0185–1.0672	0.0578
Access to food outside the home (Yes/No)	2.1599	1.0507–4.4400	0.0362
Peri-urban environment (Yes/No)	5.7043	1.9301–16.8587	0.0016
Rural environment (Yes/No)	4.6123	1.1887–17.8960	0.0271

Likelihood ratio $p < 0.0001$.

include environments with other dogs, bovines, and other small intermediate hosts, such as birds and rodents.

Acknowledgements

This work has been supported by Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-PROCAD/NF-1512/2007).

References

Barber, J.S., Trees, A.J., 1996. Clinical aspects of 27 cases of neosporosis in dogs. Vet. Rec. 139, 439–443.
Barber, J.S., Trees, A.J., 1998. Naturally occurring vertical transmission of *Neospora caninum* in dogs. Int. J. Parasitol. 28, 57–64.
Basso, W., Venturini, L., Venturini, M.C., Moore, P., Rambeau, M., Unzaga, J.M., Campero, C., Bacigalupe, D., Dubey, J.P., 2001. Prevalence of *Neospora caninum* infection in dogs from beef-cattle farms, dairy farms, and from urban areas of Argentina. J. Parasitol. 87, 906–907.
Cañón-Franco, W.A., Bergamaschi, D.P., Labruna, M.B., Camargo, L.M.A., Souza, S.L.P., Silva, J.C.R., Pinter, A., Dubey, J.P., Gennari, S.M., 2003. Prevalence of antibodies to *Neospora caninum* in dogs from Amazon, Brazil. Vet. Parasitol. 115, 71–74.
Collantes-Fernández, E., Gómez-Bautista, M., Miro, G., Álvarez-Gárcia, G., Pereira-Bueno, J., Frisuelos, C., Ortega-Mora, L.M., 2008. Seroprevalence and risk factors associated with *Neospora caninum* infection in different dog populations in Spain. Vet. Parasitol. 152, 148–151.
Costa, K.S., Santos, S.L., Uzêda, R.S., Pinheiro, A.M., Almeida, M.A.O., Araújo, F.R., McAllister, M.M., Gondim, L.F.P., 2008. Chickens (*Gallus domesticus*) are natural intermediate hosts of *Neospora caninum*. Int. J. Parasitol. 38, 157–159.
Cruz-Vazquez, C., Medina-Esparza, L., Marentes, A., Morales-Salinas, E., Garcia-Vazquez, Z., 2008. Seroepidemiological study of *Neospora caninum* infection in dogs found in dairy farms and urban areas of Aguascalientes, México. Vet. Parasitol. 157, 139–143.
Dijkstra, T., Eysker, M., Schares, G., Conraths, F.J., Wouda, W., Barkema, H.W., 2001. Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with *Neospora caninum* tachyzoites. Int. J. Parasitol. 31, 747–752.
Dubey, J.P., Hattel, A.L., Lindsay, D.S., Topper, M.J., 1988. Neonatal *Neospora caninum* infection in dogs: isolation of the causative agent and experimental transmission. J. Am. Vet. Med. Assoc. 193, 1259–1263.
Dubey, J.P., 2003. *Neospora caninum* and neosporosis in animals. Korean J. Parasitol. 41, 1–16.
Ferroglio, E., Pasino, M., Ronco, F., Bena, A., Trisciuoglio, A., 2007. Seroprevalence of antibodies to *Neospora caninum* in urban and rural dogs in north-west Italy. Zoonoses Public Health 54, 135–139.
Gondim, L.F.P., Mcallister, M.M., Pitt, W.C., Zemlicka, D.E., 2004. Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 34, 159–161.
Gondim, L.F.P., Mcallister, M.M., Gao, L., 2005. Effects of host maturity and prior exposure history on the production of *Neospora caninum* oocysts by dogs. Vet. Parasitol. 134, 33–39.
Hemphill, A., Fuchs, N., Sonda, S., Hehl, A., 1999. The antigenic composition of *Neospora caninum*. Int. J. Parasitol. 29, 1175–1188.
Jesus, E.E.V.de., Santos, P.O.M., Barbosa, M.V.F., Pinheiro, A.M., Gondim, L.F.P., Guimarães, J.E., Almeida, M.A.O., 2006. Frequência de anticorpos anti- *Neospora caninum* em cães nos municípios de Salvador e Lauro de Freitas, Estado da Bahia-Brasil. Braz. J. Vet. Res. Anim. Sci. 43, 5–10.
Katz, M.H. (Ed.), 1999. Multivariable Analysis: A Practical Guide for Clinicians. Cambridge University Press, Cambridge, 192 pp.
King, J.S., Slapeta, J., Jenkins, D.J., Al-Qassab, S.E., Ellis, J.T., Windsor, P.A., 2010. Australian dingoes are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 40, 945–950.
Kramer, L., de Risio, V.M., Tranquillo, V.M., Magnino, S., Genchi, C., 2004. Analysis of risk factors associated with seropositivity to *Neospora caninum* in dogs. Vet. Rec. 154, 392–393.
Mcallister, M.M., Dubey, J.P., Lindsay, D.S., Jolley, W.R., Wills, R.A., McGuire, A.M., 1998. Dogs are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 28, 1473–1478.
Pasquali, P., Mandara, M.T., Adamo, F., Ricci, G., Polidori, G.A., Dubey, J.P., 1998. Neosporosis in a dog in Italy. Vet. Parasitol. 77, 297–299.
Romanelli, P.R., Freire, R.L., Vidotto, O., Marana, E.R.M., Ogawa, L., De Paula, V.S.O., Garcia, J.L., Navarro, I.T., 2007. Prevalence of *Neospora caninum* and *Toxoplasma gondii* in sheep and dogs from Guarapuava farms, Paraná State, Brazil. Vet. Sci. 82, 202–207.
Wouda, W., Dijkstra, T., Kramer, A.M.H., Van Maanen, C., Brinkhof, J.M.A., 1999. Seroepidemiological evidence for a relationship between *Neospora caninum* infections in dogs and cattle. Int. J. Parasitol. 29, 1677–1682.